

## Preliminary Communication

# EVALUATION OF THE SORBENTS USED IN THE FTA-ABS TEST\*

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The fluorescent treponemal antibody (FTA) test was introduced by Deacon, Falcone, and Harris (1957). In its original form, a 1:5 dilution of the patient's serum was used. The test had a high sensitivity, but it showed considerable non-specific reactivity with non-treponemal or biological false positive (BFP) sera. Later, Deacon, Freeman, and Harris (1960) reported a modified procedure, the FTA-200 test, which used a 1:200 dilution of the serum under investigation. In this procedure the non-specific components of the test serum were expected to be diluted beyond the amount necessary to obtain a positive reaction. However, the simultaneous dilution of the specific treponemal antibody led to a decreased sensitivity of the test.

A further modification of the original method was reported by Deacon and Hunter (1962) in the form of an FTA-ABS test. As compared to the FTA-200 procedure, the new method is reported to be more sensitive since the patient's serum is diluted only 1:5 as in the original FTA procedure. It is also claimed to be more specific due to the blocking or binding of the non-specific antibodies by absorption with a substance called sorbent. The sorbent is a water-soluble extract of non-pathogenic treponemes, prepared by autoclaving the organisms in their growth medium, and it was assumed that the sorbing activity was due to an antigen-antibody binding between the Reiter antigens and the non-specific antibodies. However, the exact mode of action was not clear. Before the introduction of this sorbent, Deacon and Hunter (1962) had used a sonically-disintegrated preparation of Reiter treponemes; this method was later modified by Fribourg-Blanc and Niel (1962).

The present study was carried out to investigate the mode of action of these two types of sorbents used in the FTA-ABS test.

## Material and Methods

### Sorbents

(1) "Difco sorbent"\* was prepared according to the method described by Stout, Kellogg, Falcone, McGrew, and Lewis (1967).

(2) Reiter sonicate was prepared by ultrasonic disruption of washed Reiter treponemes as reported by Fribourg-Blanc and Neil (1962).

### Other Sorbing Substances

(1) Uninoculated Reiter treponeme medium or thio-glycollate broth (Stout and others, 1967) was autoclaved at 121°C. for 10 min. after the addition of human serum. It was then centrifuged, lyophilized, and restored with distilled water to 10 per cent. of its original volume. In other words, this sorbent was prepared by exactly the same procedure as that used for the Difco sorbent, except that no Reiter treponemes were grown in the medium.

(2) Sodium thioglycollate (0.3 per cent.) was prepared by dissolving the chemical in distilled water. This is roughly the same concentration as in the Difco sorbent.

(3) VDRL antigen emulsion was prepared by stirring the antigen mixture with 0.85 per cent. saline as in the VDRL test.

(4) *Neisseria gonorrhoeae* extract was prepared by boiling the organisms in phosphate buffer saline pH 7.2 followed by centrifugation to remove the non-soluble material.

### FTA-ABS Test with VDRL Antigen Emulsion

Since the VDRL antigen is water insoluble, it was necessary to remove it from the serum after absorption. This was done as follows.

Test serum (0.5 ml.) was mixed rapidly with 0.18 ml. of VDRL antigen emulsion in a micro-test tube (3 × 0.5 cm.) and shaken. After standing at room temperature for 15 min., the tubes were centrifuged in a micro-centrifuge (O. Dich Co.) for 2 min. at 20,000 r.p.m. The supernatant was gently separated from the sedimented precipitate and mixed with 1.82 ml. phosphate buffered saline (pH 7.2) to yield a 1:5 dilution of the original serum. The FTA test was performed on this pre-absorbed serum sample without further dilution or treatment.

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**FTA-ABS Test**

The procedure described by Deacon and Hunter (1962) was followed in the FTA-ABS tests with all the sorbents, excepting the VDRL antigen emulsion, because the sera were already pre-absorbed.

**Results**

Table I summarizes the information on 55 human sera which were investigated. They were selected on the basis of their reactivity in the FTA-5 test. Serum category was based on clinical information and on the results of the TPI and the cardiolipin-complement fixation (WR) tests.

TABLE I  
INFORMATION ON SERA INVESTIGATED

Serum Category	Number of Sera	Range of Fluorescence (FTA-5)			
Normal	10	+	→	+++	
Syphilitic	25	+++	→	++++	
Yaws	8	++	→	+++	
BFP	12	++	→	+++	

**Discussion**

There are two basic assumptions underlying the FTA-ABS test, *viz.*:

(1) The reactivity of most normal sera in the FTA-5 test is due to the presence of antibodies to the non-pathogenic treponemes, such as *Treponema microdentium*, which often exist in the human oral cavity.

(2) These non-specific antibodies are absorbed or blocked by the Reiter antigens present in the sorbent.

The group-specific antibodies are directed towards antigens shared by both the pathogenic and the non-pathogenic treponemes, and therefore they are non-specific as far as syphilitic infection is concerned. If the reactivity of normal sera in the FTA-5

test is caused by the presence of non-specific antibodies, the latter could be detected in the Reiter protein complement-fixation test (RPCF) or in the haemagglutination test utilizing Reiter protein as the antigen. However, non-syphilitic sera which were reactive in the FTA-5 test were not reactive in the RPCF or the haemagglutination tests.

The false reactivity of normal sera in the FTA-5 test may be caused by the binding of the treponemes and certain serum components, such as anti-lipoidal factors or macroglobulins, that are produced in response to a stimulus other than treponemal infection.

Since the uninoculated Reiter medium lowered the degree of fluorescence with normal, FTA-5 reactive sera, one cannot attribute the sorbent activity to the immune binding of the non-specific antibodies by the Reiter antigens. Similar findings have been reported by Cannefax and others (1968), who also investigated the sorbing activity of each component of the Reiter medium.

Since the sulfahydryl group (SH) containing agents, such as sodium thioglycollate or cysteine HCl, reduce the macroglobulins, it was of interest to see whether these components of the Reiter medium contributed to the sorbing activity by affecting some of the macroglobulins.

Immuno-electrophoretic studies on a syphilitic serum containing macroglobulins showed no change in the immuno-electrophoretic pattern of the latter after incubation for 30 mins with the Difco sorbent. However, a slight change in the immuno-electrophoretic pattern was seen only after a 5 hour incubation period.

Sodium thioglycollate (pH 5.85) was investigated for sorbing activity at the concentration present in the Difco sorbent. The results in Table II show that the degree of fluorescence was decreased, though not to the same extent as with the Difco preparation.

TABLE II  
COMPARISON OF RESULTS WITH DIFFERENT SORBENTS IN FTA-ABS TEST

Serum Category	Number of Sera	Degree of Fluorescence						
		Before Absorption	Difco	Reiter Medium	Reiter Sonicate	Na Thioglycollate	VDRL Antigen	<i>N. gonorrhoeae</i>
Normal	1 2	+	—	—	—	—	—	—
		+++	—	+	+	+	+	—
Syphilitic	1 2	+++ ++++	+++ ++++	+++ ++++	+++ ++++	+++ ++++	+++ ++++	+++ ++++
Yaws	1 2	+++ +++	++ ++	+	++ +++	++ +++	++ +++	not done not done
BFP	1 2	++ +++	— +	—	—	+	—	—

This Table presents the results of the FTA-ABS test with six different sorbing substances. Results on eight sera, including representative samples from each category, are given.

The sorbing activity of the sodium thioglycollate solution disappeared when the pH of the solution was adjusted to 7.2, suggesting that the sorbing effect is pH-dependent. The pH of the Difco sorbent and of similar preparations containing concentrated Reiter medium was found to be about 5.8, which is considerably lower than the optimal pH of 7.2 for the test. Although the original medium has a pH of 7.2 before inoculation, the final pH of the sorbent is 5.8 possibly due to cultivation of the treponemes, autoclaving, and several-fold concentration of the ingredients. Since the test serum is diluted five times with the sorbent, the final pH of the mixture is still much lower than the optimal pH of 7.2 which is used in the control test with the buffer.

The sorbing effect was found to be not only pH-dependent but also concentration-dependent, since increasing the concentration of the sorbent or using 0.8 per cent. thioglycollate decreased the degree of fluorescence to a greater extent.

The sorbing activity of Reiter medium-containing sorbents may also be due to other factors besides low pH and concentration of the ingredients. The overall effect is possibly a reduction of the affinity between the antigen and the specific as well as the non-specific serum components. This reduction in affinity is then manifested in the production of a lower degree of fluorescence.

Reiter sonicate is free from any traces of the medium and, when used as a sorbent it cannot be expected to alter the optimal conditions of the test. Here one can envisage an immunological binding of the non-specific antibodies by the Reiter antigens. However, similar sorbing activity by an extract of *Neisseria gonorrhoeae* raises doubts against this possibility. It is likely that some of the globulin components of the serum are removed by physical absorption when the sorbent consists of concentrated mixtures of phospholipids, or extracts of yeast or other micro-organisms.

VDRL antigen was used as the sorbent to absorb from syphilitic and BFP sera the antilipoidal factors which are known to interfere in various serological reactions. The results shown in Table II indicate that the VDRL antigen was able to remove certain serum components resulting in a non-reactivity of the normal and BFP sera.

The use of this antigen as a sorbent seems satisfactory since it is a mixture of pure, chemically well-defined substances and it is precipitable. Its removal produced no apparent change in the concentration of the specific antibodies. After four cycles of treatment with the VDRL antigen emulsion, a syphilitic serum was non-reactive in the WR test but still

strongly reactive in the TPI test, indicating removal of the non-specific serum components without changing the concentration of the specific antibodies to a great extent.

It is not the intention of this report to recommend VDRL antigen as the ideal sorbent in the FTA-ABS test. However, investigation with a larger number of sera may help to understand the basis of the test.

Whatever the mechanism involved in the action of sorbents in the FTA-ABS test, the desirable result of suppressing only the non-specific antibodies seems to be achieved, but this effect may be only apparent rather than real since some of the factors responsible for sorbent activity can affect the specific antibodies as well. In most syphilitic sera the concentration of the latter is quite high and it is not greatly affected by partial suppression. Also, if the concentration of the non-specific factors is high in some BFP sera, sorbent activity is not adequate to remove these factors completely.

The FTA-ABS test has been used to diagnose some cases of ocular syphilis and neurosyphilis (Smith and Taylor, 1965), which were non-reactive in other tests, including the TPI test. It is always risky to depend upon one serological test for the final diagnosis, particularly so with the FTA-ABS test, the specificity of which is not well established.

The FTA-ABS test has several practical advantages and the test has gained considerable popularity. However, results reported by Cannefax and others (1968) and in this publication raise considerable doubt as to the specificity of the test.

### Summary

The FTA-ABS test was performed on 55 human sera, including normal, syphilitic, and BFP specimens, with currently-used sorbents. Other substances, such as sodium thioglycollate, concentrated Reiter medium, VDRL antigen, and an extract of *Neisseria gonorrhoeae*, were also used as sorbing agents.

Factors, such as low pH, concentration of the ingredients, especially reducing agents, and physical absorption of the serum components by the sorbent, may be partially responsible for the decrease in the degree of fluorescence.

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**L'évaluation des agents sorbiques dans le test  
FTA-ABS  
Communication préliminaire**

## RÉSUMÉ

Le test FTA-ABS a été fait avec 55 sérums humains, y compris des spécimens normaux, syphilitiques et BFP, en se servant des agents sorbiques couramment en usage. D'autres substances, telles que l'hypoglycolate de soude, l'antigène VDRL concentré du milieu de Reiter, et un extrait de *Neisseria gonorrhoeae*, ont été aussi employées comme agents sorbiques.

Des facteurs, tels que le pH bas, la concentration des ingrédients, spécialement les agents réducteurs, et l'adsorption physique des parties constitutantes du sérum par l'agent sorbique peuvent être partiellement responsables de la diminution du degré de fluorescence.